

Effect of polyamines and zeolites on the protein profile of leaves of the date palm cuttings *Phoenix dactylifera* L. grown under heavy metal stress conditions

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This study was conducted on the five to six years old offshoots of date palm (Jebjab) cultivar planted in sustainable land, using an environment contaminated with heavy elements, with irrigation water contaminated with heavy elements $\text{Pb}(\text{NO}_3)_2$ 300 mg.kg⁻¹ and cadmium chloride CdCl_2 3 mmol, as well as the comparison treatment. Then the treatment was with some polyamine compounds Putrescine 500 mg L⁻¹, coumaric acid 500 mg L⁻¹, and a zeolite compound at a rate of 10 kg Palm⁻¹, in addition to the comparison treatment in the form of ground addition to the soil of the off shoots. Samples were taken from palm fronds, samples were dried using Freeze-dryer (Lyophilization technique), the protein was extracted from samples, and protein migration was carried out on Polyacrylamide gel using Slab-Electrophoresis method in the presence of SDS denaturants. The molecular weights of proteins were estimated and plotted using PhotoCapt Mw. The results of the study showed that the protein pattern of the leaves show up that all the trees of the study had taken part (identical) with the first protein bundle and the fifth protein bundle on the polyacrylamide gel, as the molecular weights of the proteins of the first bundle ranged between (222.136-243.750) kDa, while the molecular weights of the proteins of the bundle ranged. The fifth is between (24.843-50.386) kDa. This coincidence or participation in the molecular weights of the protein bundles of all the study trees may indicate that these trees belong to one plant origin (which is the Jebjab variety), as these plants share close molecular weights.

Keywords: Date Palm, molecular weights, polyacrylamide gel, polyamine compounds.

INTRODUCTION

The date palm *Phoenix dactylifera* L belongs to the palm family Arecaceae. This family includes more than 4000 species and nearly 200 genera (Al-Jubouri,2002). The date palm is one of the most important evergreen fruit trees in several countries of the world. Iraq and the Arabian Gulf region are believed to be its original home. It is possible that it originated in southern Iraq. Since ancient times, Iraq was known for palm cultivation, production, and marketing of dates in the world (Bhat AL-Daihan, 2012; Rasheed and AL-Badri, 2018). Date palm trees in Iraq have been subjected to a significant decrease in their production levels and a severe shortage in their numbers during the past two decades (Central Statistical Organization, 2021).

Pollution is defined as any undesirable change in the physical, chemical, or vital properties of the environment (air, water, and soil), which occurs as a result of pollution-causing

substances called pollutants, which are any solid, liquid, or gaseous substance found in certain concentrations that are transmitted from different sources and in different quantities (Bhatia, 2009; Al-Wahaibi, 2007). The toxicity of heavy elements has become one of the important and dangerous phenomena and has harmful effects on the morphological form and the decrease in the rate of photosynthesis in the plant due to the closure of stomata as a result of the deposition of elements and the inhibition of enzymatic activities and an imbalance in the water balance as well as affecting the permeability of cell membranes in addition to causing a disturbance in the absorption of nutrients (Kabir *et al.*, 2010). Heavy elements such as lead and cadmium are among the most dangerous elements that pollute soil and water, and the presence of these elements in the soil leads to a decrease in leaf area and a decrease in chlorophyll production, which leads to inhibition in the photosynthesis process and then a decrease in carbohydrates (Singh *et al.*, 2011, Aljaberi, *et al.*,

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2020). And adding cadmium and lead to the soil leads to a significant decrease in protein concentration (Khodaverdiloou *et al.*, 2011, Alhasany, *et al.*, 2020). Exposure of plants to stress of heavy elements is likely to cause phytotoxicity that leads to delayed seed germination, reduced nutrient absorption, disturbances in metabolism, and then weakness in plant growth and development, deterioration of production, and a decrease in the ability to fix molecular nitrogen (Ashraf and Ali, 2007, Alhasany, *et al.*, 2021).

Using natural conditioners improves the soil's physical, chemical, and fertility properties, and thus provides a good environment for plant growth in terms of increasing moisture content and providing nutrients, the most important of which is zeolite. The treatment of plants with polyamine compounds such as aspirin, putrescine, and coumaric acid improved the activity of enzymatic antioxidants and the concentrations of non-enzymatic antioxidants, as well as the concentrations of osmotic buffers such as sugars, proline, and others (Yamada *et al.*, 2002).

Due to the increasing rates of heavy metal pollution, these studies have tended to use polyamines to alleviate abiotic stresses such as heavy metal toxicity to maintain normal growth and economic productivity.

MATERIALS AND METHODS

This study was conducted on the five to six years old offshoots of date palm (Jebjab) cultivar planted in sustainable land, using an environment contaminated with heavy elements, with irrigation water contaminated with heavy elements Pb (NO₃)₂ 300 mg.kg⁻¹ and cadmium chloride CdCl₂ 3 mmol, as well as the comparison treatment. Then the treatment was with some polyamine compounds Putrescine 500 mg L⁻¹, coumaric acid 500 mg L⁻¹, and a zeolite compound at a rate of 10 kg Palm⁻¹, in addition to the comparison treatment in the form of ground addition to the soil of the offshoots. Samples were taken from palm fronds and dried by freeze-dryer (lyophilization technique) at a temperature of -26°C. Protein was extracted from the samples according to the method described in (Al-Najjar *et al.*, 2021) by taking 1 g of wicker and placing it in a ceramic mortar with 3 ml of Tris-HCl-buffer (0.1M, pH7.5) containing (PMSF) Phenyl methane sulfonyl fluoride on At a temperature of (4°C), then the centrifugation process was carried out at a temperature of (4°C) and a speed of (18000 R/m) revolution per minute for half an hour, then (40 microliters) of the filtrate was taken to the migration device on a Polyacrylamide gel.

Protein migration was carried out on a polyacrylamide gel using the Slab-Electrophoresis method in the presence of SDS denaturants according to the method described by Bavei *et al.* The markers were used (Broad Range Protein Molecular Weight Markers) from Promega, and the molecular weights of the proteins were estimated and plotted using a special computer program, PhotoCapt Mw (17version).

The coefficients were numbered on the gel image with the following numbers: Column 1 =marker / Column 2=control / Column 3=cd+put / Column 4=pb+zu / Column 5=pb+co / Column 6=pb+put / Column 7=put / Column 8=cd+zu / Column 9=cd+co / Column 10= pb / Column 11=cd / Column 12=zu / Column 13 = co

RESULTS AND DISCUSSION

From observing the results of the protein pattern of the leaves of the date palm offshoots of the Al-Jibjab cultivar under study (Fig. 2-a-b-c-d-e), we find that there are differences between all the study conditions, as the specifications of the protein bundles differed in terms of size, area, and height according to the treatments which effects in specifications of protein bundles.

The results of the proteolytic pattern of the leaves of the date palm offshoots of the Gijab variety under study (Fig. 3) show that all the studied trees shared (identical) with the first and the fifth protein band on the polyacrylamide gel, as the molecular weights of the first band proteins ranged between (222.136-243.750). kilo Daltons. While the molecular weights of the proteins of the fifth band ranged between (24.843-50.386) kilo Daltons. This coincidence or sharing of the molecular weights of the protein bundles of all the trees of the study may indicate that these trees belong to one plant origin (which is the Jebjab variety), as these trees shared plants with similar molecular weights.

The results of the proteolytic pattern of the leaves of the date palm offshoots of the Gijab variety under study (Fig. 3) show that there are differences between these trees in the number, locations, and specifications of the protein bundles on the polyacrylamide gel. The number of protein bundles ranged between (6-10) protein bundles depending on the Treatment type, it was six protein bundles in each of the overlapping treatment (lead with putrescine) and the overlapping treatment (cadmium with coumaric acid), while it appeared in most of the treatments (comparison, cadmium with putrescine, lead with zeolite, lead with coumaric acid, tyrosine, cadmium With zeolite, zeolite, and coumaric acid) seven protein bundles, as when observing these treatments (treatment with bitcoin, coumaric acid, and zeolite and their interactions with pollution treatments), we find that these compounds (pollution treatments) when they interfered with lead and cadmium prevented the change of the protein pattern of trees, as the number The routine packets have an equal number of protein packets for the comparison treatment.

It is noted from the migration results of the proteins on the polyacrylamide gel that there are ten protein bundles in each of the lead contamination treatment and the cadmium contamination treatment, with closeness or conformity in the molecular weights of the proteins in each of those bundles for both treatments.



Also the study treatments had a clear effect on the gene expression process of the trees and caused the emergence of new protein bundles that reached ten bundles (as in the treatment of lead and cadmium contamination), as this indicates that the study treatments have stimulated the cells to manufacture new proteins that support Indicators of growth and development of growing plants under the stress of pollution with heavy elements. The treatments also had a clear effect in changing the locations of protein packages and their different molecular weights, which indicates that the treatments have caused activation of the gene expression process and the manufacture of new proteins that may have a role in improving plant growth and resistance to pollution. These results indicate that treating plants with pollutants may lead to the synthesis of natural proteins, as well as a change in the translation and transcription processes, which leads to the production of new proteins through the gene expression process according to the plant's need and in response to the type of treatment to ensure the improvement of plant growth (David and Nilson, 2000 ; Khairallah, 2009).

Figure (1): The polyacrylamide gel shows the protein bands of the study parameters.

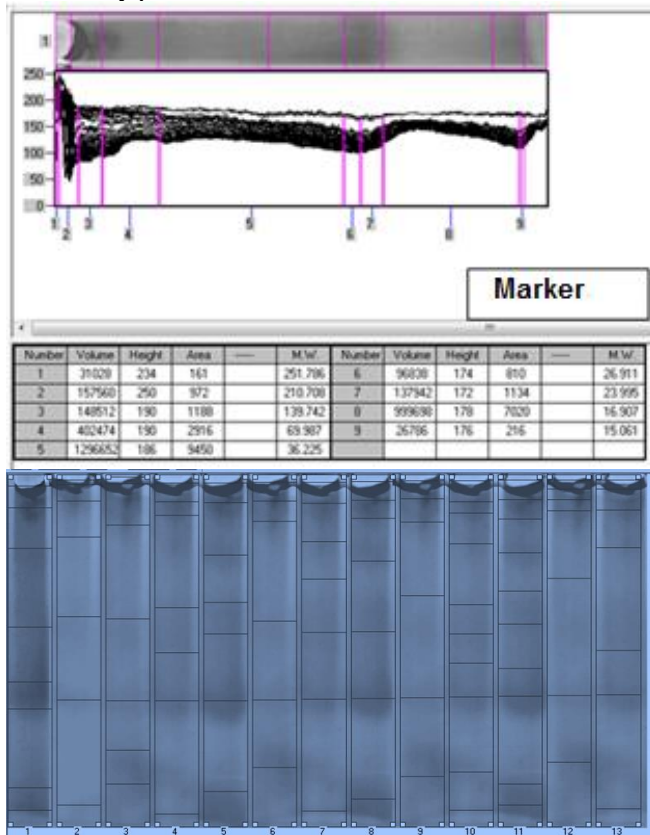


Figure (2-a). Some specifications of the protein bands on the polyacrylamide gel for the marker.

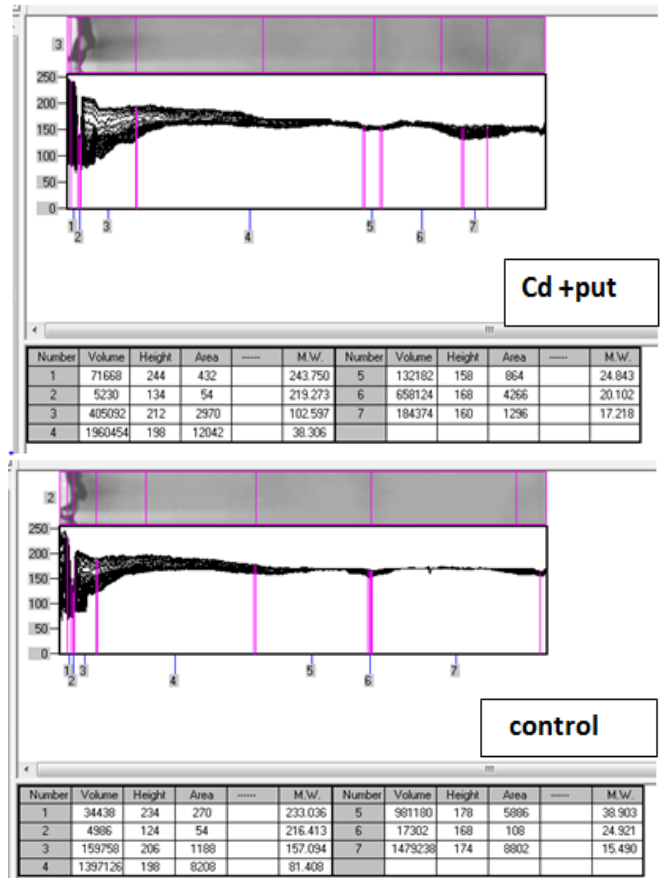


Figure (2-b). Some specifications of protein bands on polyacrylamide gel

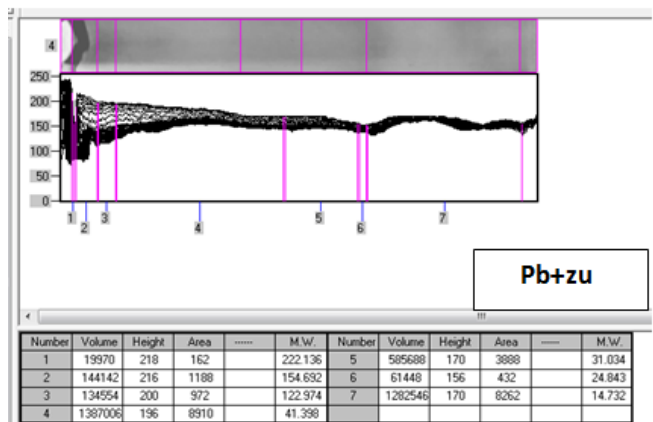


Figure (2-b). Some specifications of protein bands on polyacrylamide gel.



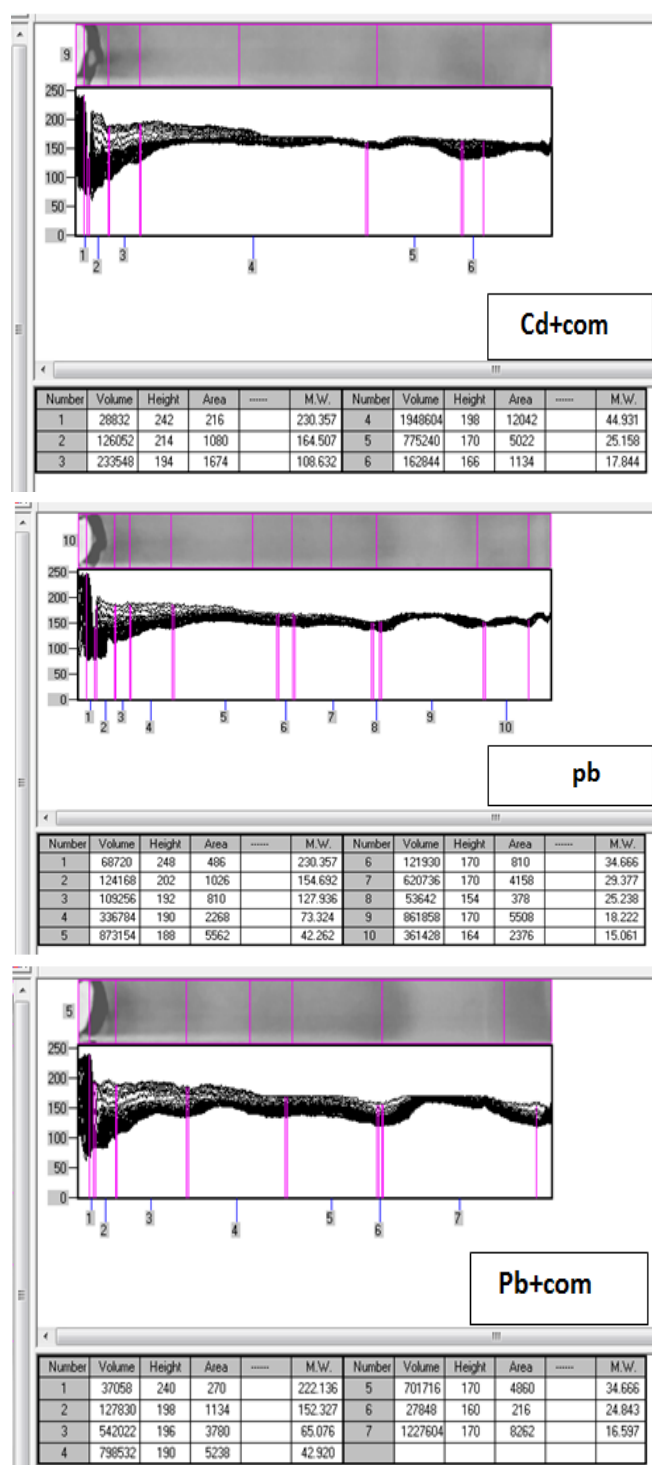


Figure (2-c). Some specifications of protein bands on polyacrylamide gel.

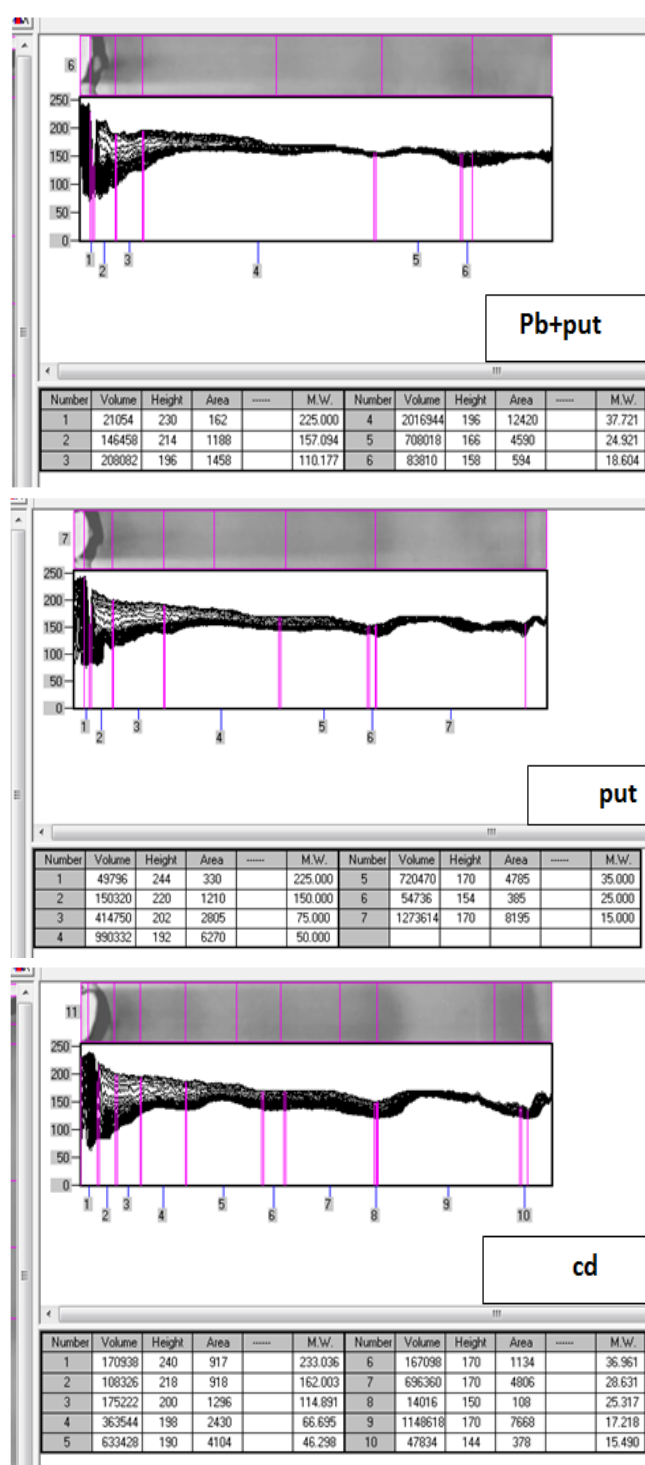


Figure (2-d). Some specifications of protein bands on polyacrylamide gel.



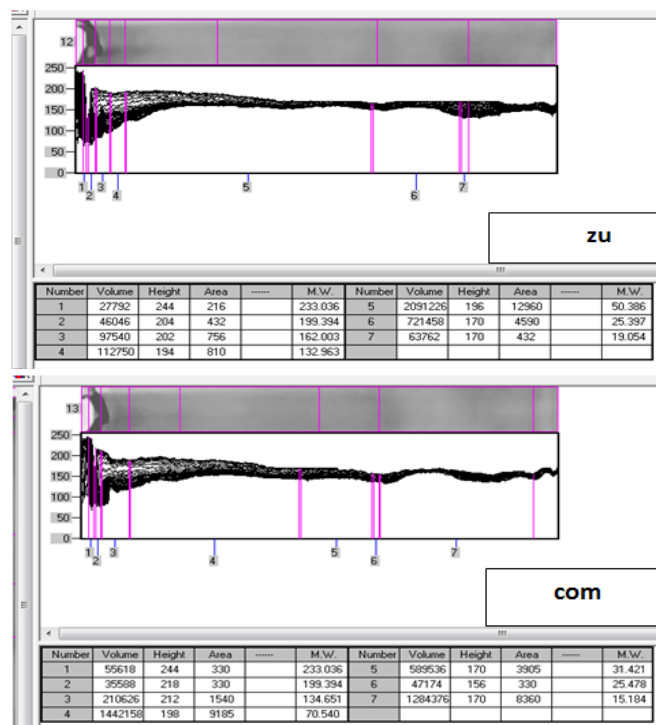


Figure (2-e) Some specifications of protein bands on polyacrylamide gel

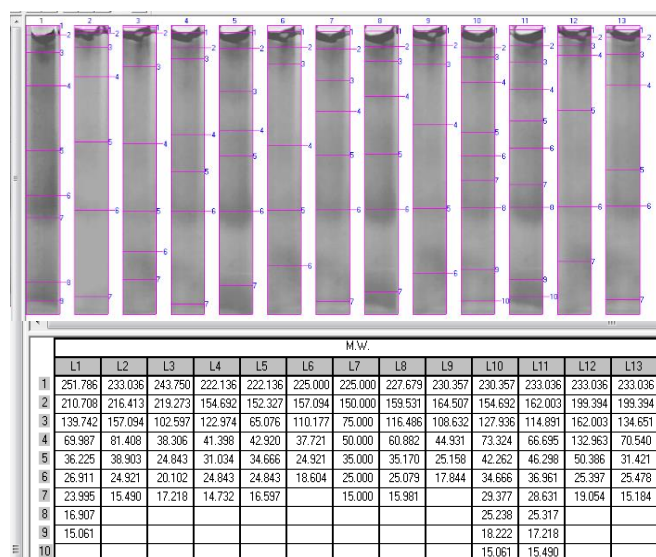


Figure 3. The number and locations of protein packages and their molecular weights (a side of the Photocapt program). Column 1 =marker / Column 2=control / Column 3=cd+put / Column 4=pb+zu / Column 5=pb+co / Column 6=pb+put / Column 7=put / Column 8=cd+zu / Column 9=cd+co / Column 10= pb / Column 11=cd / Column 12=zu / Column 13 = co

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